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Proceedings of the National Peer Review on Environmental and Indirect Human Health Risk Assessments of the GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra

September 12-14, 2017
Ottawa, Ontario

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Foreword

The purpose of these Proceedings is to document the activities and key discussions of the meeting. The Proceedings may include research recommendations, uncertainties, and the rationale for decisions made during the meeting. Proceedings may also document when data, analyses or interpretations were reviewed and rejected on scientific grounds, including the reason(s) for rejection. As such, interpretations and opinions presented in this report individually may be factually incorrect or misleading, but are included to record as faithfully as possible what was considered at the meeting. No statements are to be taken as reflecting the conclusions of the meeting unless they are clearly identified as such. Moreover, further review may result in a change of conclusions where additional information was identified as relevant to the topics being considered, but not available in the timeframe of the meeting. In the rare case when there are formal dissenting views, these are also archived as Annexes to the Proceedings.

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SUMMARY

The *Canadian Environmental Protection Act* (CEPA), administered by Environment and Climate Change Canada (ECCC) and Health Canada (HC), is the key authority for the Government of Canada to ensure that all new substances, including living organisms, are assessed for their potential harm to the environment and human health. ECCC and HC are responsible for conducting a CEPA risk assessment to evaluate whether an organism product of biotechnology is "CEPA toxic" in accordance with Section 64 of CEPA.

In accordance with a Memorandum of Understanding between Fisheries and Oceans Canada (DFO), Environment and Climate Change Canada (ECCC), and Health Canada (HC), DFO assists in implementing the *New Substances Notification Regulations (Organisms)* [NSNR(O)] by providing science advice that is based on an environmental risk assessment for fish products of biotechnology, and, with the support of HC, on the indirect human health risk assessment for fish products of biotechnology. DFO may also make recommendations regarding any necessary measures to manage risks, if required. DFO provides the advice as a Science Advisory Report through the Canadian Science Advisory Secretariat (CSAS) National Review Process.

On July 5, 2017, a regulatory notification was submitted to ECCC for the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra (CGT2016), a genetically engineered tropical fish species (Black Tetra *Gymnocorymbus ternetzi*) notified by GloFish LLC under the NSNR(O). The notifier intends to import CGT2016 to Canada for sale as an ornamental aquarium fish. DFO and HC collaborated to conduct the environmental and indirect human health risk assessments, respectively, and to develop recommendations to support a regulatory decision by the Minister of Environment and Climate Change. The purpose of the CSAS Science National Review Process was to undertake a national peer review of the risk assessment and to develop scientific consensus on the risk assessment outcomes and recommendations provided to ECCC, which, in collaboration with HC, is responsible for the final CEPA Risk Assessment. The peer review meeting was held on September 12-14, 2017 in Ottawa, Ontario. The terms of reference and agenda for this process are found in Appendix 1 and 2, respectively. Meeting participants included experts, science advisors, and research scientists from ECCC and HC, various sectors and regions of DFO, and from the University of Ottawa, University of New Brunswick, and St Michael's Hospital (Appendix 3). The conclusions and advice resulting from this meeting are provided in the form of a Science Advisory Report, as well as two peer-reviewed risk assessment documents that will be publically available on the CSAS website.

INTRODUCTION

On July 5, 2017, a regulatory package was submitted by GloFish LLC to Environment and Climate Change Canada (ECCC) under the *New Substances Notification Regulations (Organisms)* [NSNR(O)] of the *Canadian Environmental Protection Act* (CEPA) for the GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra (CGT2016). In accordance with a Memorandum of Understanding between Fisheries and Oceans Canada (DFO), ECCC, and Health Canada (HC), DFO assists in implementing the NSNR (Organisms) by providing science advice based on an environmental risk assessment for CGT2016, and, with the support of HC, on the indirect human health risk assessment of CGT2016. This advice supports a regulatory decision by ECCC and HC for CGT2016.

The Science National Review process included participants with relevant expertise to review and discuss the draft "Environmental Risk Assessment of the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra: Transgenic Ornamental Fish, Imported to Canada, For Sale in the Pet Trade" prepared by DFO, and the draft "CEPA Human Health Assessment Report on *Gymnocorymbus ternetzi* CGT2016" prepared by HC (herein after referred to as the environmental risk assessment and indirect human health (IHH) risk assessment, respectively). There was representation from a variety of divisions of DFO, ECCC, and HC, as well as an external panel of academic experts in transgenics (University of Ottawa), fish physiology (University of New Brunswick), and zoonosis (St. Michael's Hospital, Toronto). Participants were provided with the draft risk assessments in advance and contributed comments for consideration prior to the meeting. Subsequently, a face-to-face "Canadian Science Advisory Secretariat (CSAS) National Peer-Review Process for the Environmental and Indirect Human Health Risk Assessments of the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra" meeting was held September 12-14, 2017 in Ottawa, Ontario. Discussion at this meeting focused on the main components of the two draft risk assessments including exposure assessment, hazard assessment, and associated levels of uncertainty. Consensus was reached on the document output of the meeting; a draft Science Advisory Report entitled "Environmental and Indirect Human Health Risk Assessment of the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra (*Gymnocorymbus ternetzi*): A Transgenic Ornamental Fish". This Science Advisory Report was submitted to ECCC as final science advice to support ECCC's CEPA risk assessment and regulatory decision of ECCC for the notified organism.

CSAS SCIENCE NATIONAL REVIEW PROCESS

Presenter: Gilles Olivier, Chair; Fisheries and Oceans Canada

The meeting chair, Gilles Olivier (DFO – National Capital Region) provided an overview of the "CSAS Science National Peer-Review Process", the principles of CSAS, and explained the role of all meeting participants as reviewers. He emphasized the strictly scientific basis of consensus on CSAS processes, as well as the ground rules for the meeting and expected publications.

CSAS provides science advice in support of DFO policy, and management plans and decisions. The approach is based on the SAGE (Scientific Advice for Government Effectiveness) principles and guidelines for the effective use of science and technology advice in government decision making. The main objectives are to provide sound, objective, and impartial science advice. Participation in the CSAS process is by invitation to those with the expertise and knowledge on the subject matter. Scientific working paper(s) and other inputs (analysis, findings, conclusions) are subject to rigorous review and quality control in a peer-based forum. The resultant peer-reviewed documents are released to the public and the final Scientific Advisory and Response

Reports, Research Documents (Risk Assessments), and Proceedings are published on the CSAS website.

All participants were asked to review the material provided, participate fully in discussion, contribute relevant expertise, experience, data, and knowledge on the subject of review and consider the application of the data and methods. It was noted that participants should work toward developing consensus on the conclusions and advice.

In response to questions from participants, the following clarifications were provided:

- Although the documents are released to the public, there is a confidentiality agreement with GloFish LLC (hereafter referred to as the notifier). The notifier would be provided with an opportunity to identify any confidential information in the Risk Assessment, Science Advisory Report, and Proceedings that would need to be redacted.
- According to the Terms of Reference, the specific document endpoints are 1) the Environmental Risk Assessment, 2) the Indirect Human Health Risk Assessment, and 3) the Science Advice Report (SAR).
- With regard to the SAR, participants would write a series of bullets in the Summary section of the Science Advisory Report toward the end of the meeting. The SAR essentially is informed by and is a summarized version of the two risk assessments. After the bullets are written, participants together would review the SAR on screen, and contribute comments on the main body. After the meeting convened, participants were asked to provide edits to DFO, and then the final revised copy would be circulated for consensus within two weeks after the meeting close.

REGULATORY CONTEXT, RISK ASSESSMENT PROCESS, AND PROPOSED USE SCENARIO SUMMARY

Presenter: Colin McGowan, Fisheries and Oceans Canada

The "Regulatory Context, Risk Assessment Process, and Proposed Use Scenario Summary" presentation addressed the legislative and regulatory context under which the risk assessments were conducted, the risk assessment process itself, and the translation of the risk assessment findings into a recommendation (i.e., the Science Advisory Report) for a regulatory decision under CEPA, given the proposed use scenario for the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra.

It was noted that this is not a typical risk assessment under the *Fisheries* Act as here the overall responsibility falls to ECCC and HC. The risk assessments were conducted under CEPA, which is an act respecting pollution prevention and the protection of the environment and human health in order to contribute to sustainable development. The biotechnology provisions of CEPA take a preventative approach to pollution by requiring all new living organism products of biotechnology, including genetically engineered (GE) fish, to be notified and assessed prior to import or manufacture. As such, ECCC and HC are responsible for conducting the CEPA risk assessment to evaluate whether the notified fish product of biotechnology is "CEPA toxic" in accordance with Section 64 of CEPA 1999. As defined in section 64 of CEPA, an organism is "toxic" if it is entering or may enter the environment in a quantity or concentration or under conditions that:

1. have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
2. constitute or may constitute a danger to the environment on which life depends; or,

3. constitute or may constitute a danger in Canada to human life or health.

Anyone proposing to import or manufacture a living animal product of biotechnology in Canada, including a GE fish, is required to provide ECCC with the information prescribed in Schedule 5 of the NSNR(O) at least 120 days prior to the commencement of import or manufacture of the organism. A waiver for one or more regulatory information requirements specified in Schedule 5 of the NSNR(O) may be requested by the applicant. Then, under a Memorandum of Understanding (MOU) with ECCC and HC, DFO provides science advice in the form of an environmental risk assessment, and works with HC on an indirect human health risk assessment, for fish products of biotechnology under the NSNR(O).

In this process, the risk assessment framework involves problem formulation, which defines the scope of the risk assessment, a description of procedures for concluding on risk, a characterization of the organism and comparator species, as well as a characterization of the ecological parameters and receiving environment. Both the environmental and IHH Risk assessments are comprised of two main parts, an exposure assessment wherein the likelihood of occurrence in the environment and likelihood of environmental contact with humans is evaluated, and a hazard assessment wherein the magnitude of ecological consequences and impacts to human health through environmental exposure (indirect) are evaluated.

Both risk assessments are conducted under the classic paradigm where:

Risk = Hazard × Exposure

In general, for each endpoint (e.g., toxicity, potential to act as vector for human zoonoses), hazard and exposure are ranked as: negligible, low, moderate, or high, and the uncertainty associated with both the exposure and hazard assessments are ranked as: negligible, low, moderate, or high. Differences between HC and DFO in the use and number of exposure rating levels were acknowledged and discussed. In this particular assessment, HC used only three categories for exposure and hazard ratings (low, medium, high), whereas DFO used four categories (negligible, low, medium, high). Also, HC uses mid-categories, e.g., low-medium, whereas DFO selects the highest of two possible categories as a final exposure level rating.

A peer review of the risk assessments is then conducted under the DFO Canadian Science Advisory Secretariat, and DFO provides conclusions of risk assessment as Science Advice to ECCC and HC. A final regulatory decision is made by the Minister of ECCC, who must decide whether the notified organism is "not CEPA toxic", "not CEPA toxic for the proposed use", or "CEPA toxic". If found "not CEPA toxic", no further action is required from government, though eligibility for the Domestic Substances List (DSL) will depend solely on whether the criteria of s.112(1) of CEPA are met. If "not CEPA toxic for the proposed use but suspected or capable of being toxic for other potential uses", a Significant New Activity (SNAc) notice will be issued. If found "CEPA toxic" or capable of becoming "CEPA toxic", then the manufacture or import of the organism may be subject to conditions, there may be a complete prohibition of the product, or there may be no import or manufacture of the organism until additional information or data regarding the organism is provided by the notifier.

In response to questions from participants, the following clarifications were provided:

- CGT2016 has been approved for sale in United States (US) by the Food and Drug Administration; however, Canadian officials have no access to that information.
- Though the original GloFish® (a fluorescent Zebrafish) was cleared for use in the US in 2003, use of GloFish® in the ornamental trade was banned from California until 2015.
- If there is missing information, ECCC can request it from the notifier.

- Regarding the Regulatory Decision process, the CSAS process reviews the two Risk Assessments based on science, and these in turn are used to inform the CEPA risk assessment and Regulatory Decision by ECCC and HC.

CHARACTERIZATION OF GLOFISH® ELECTRIC GREEN® TETRA AND GLOFISH® LONG-FIN ELECTRIC GREEN® TETRA

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The "Characterization of CGT2016 GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra" addressed the molecular structure and function of the transgene, strain propagation, the phenotype, and off-target phenotypes. It also considered the comparator species *Gymnocorymbus ternetzi* (Black Skirt Tetra, or Black Tetra), its history of use in the aquarium trade and associated pathogens. Surrogate models with transgenes causing fluorescence, such as red fluorescent protein (RFP) in Zebrafish *Danio rerio* were also taken into consideration.

The construct was assembled through standard molecular biology techniques, did not include toxic sequences or mobile genetic elements, and was concluded unlikely to cause harm to the environment or indirect human health. Should transcription of one gene fail to terminate, the two fluorescent transgenes are oriented in the same direction, preventing the possibility of transcribing a functional region in the opposite direction.

To generate CGT2016, the transgene construct was injected into newly fertilized eggs of a wild-type white variant of the Black Tetra (*G. ternetzi*, hereafter referred to as White Tetra). A single founding individual was identified by green fluorescence, and separately crossed to several non-transgenic White Tetras to produce several F1 groups. Ultimately, six F3 fish were used to create the CGT2016 line after confirmation of a single insert copy and site for the construct by quantitative PCR and Southern blot analysis, and observed Mendelian segregation frequencies during breeding.

The target phenotypic effect was a green appearance under ambient light, and fluorescence green under blue or UV light. Two off-target phenotypic effects identified by GloFish LLC in CGT2016 are diminished tolerance to low temperature and decreased reproductive success in competition relative to wild-type siblings. CGT2016 has been in commercial production for the ornamental aquarium trade in the US since 2012.

Much of the information available on the wild-type Black Tetra is from the ornamental aquarium trade, where it has been used since at least 1950. Ornamental escapes of Black Tetra have become established in Colombia, and isolated occurrences without long-term establishment have been reported in Florida, Louisiana, and in a hot spring in Colorado.There are no other known reports of occurrences or establishments of escaped Black Tetras during its use in the ornamental aquarium trade. Behaviourally, Black Tetras are generally not known to be aggressive to or highly competitive with other aquarium fish.

Though no formal studies have compared potential disease susceptibility of CGT2016 and wild-type strains, GloFish LLC provided veterinarian statements that state no evidence for increased susceptibility to, or transmission of, water-borne pathogens, or additional health impediments of CGT2016.

DISCUSSION

Discussion after the presentation identified that the legislative and regulatory recourse for "improved" versions of the notified construct or new gene constructs (e.g., to create new colour

of fish, make a brighter green) would be for the notifier to submit a re-notification to ECCC and go through the CSAS peer-review process again.

Participants expressed concerns about the lack of data on the DNA sequence of flanking regions. This information would clarify that no endogenous genes are disrupted. It is possible that insertion of the transgene in certain regions of the genome may have potential to alter locus structure. Additionally, the point was made that the test to confirm no integration of plasmid pieces in the organism used primers that did not amplify adjacent to the junction of the plasmid and transgene construct, and would not have been detected. This information gap is something that could be investigated through additional sequencing.

Participants expressed concerns about off-target effects and potential phenotypic instability that may cause issues when hobbyists breed CGT2016. Individuals homozygous for silenced transgenes could be free from off-target effects that may be limiting fitness, and hence survive longer at Canadian temperatures and with higher breeding success. There has been no research on gene silencing through extensive multi-generational crosses, and the notifier cannot confirm phenotypic stability. If gene silencing does occur, home bred CGT2016 could go undetected for a generation, or more. However, the limited cold tolerance of CGT2016 and wild-type Black Tetra makes this issue less relevant in Canadian waters.

In response to questions from participants, the following clarifications were provided:

- Unlike the US, in Canada, anyone is legally permitted to intentionally breed the fish at home. Patents in the US do not allow intentional breeding and/or any sale, barter, or trade of any offspring of GloFish®, but in Canada, a patent is only permitted for the construct itself, not for the living organism.
- There is no information on the phenotypic reaction norm or heritable phenotypic plasticity of the notified organism or its comparator. Therefore, if fish do manage to breed in the wild, offspring may, in rare cases, be able to adapt to the local environment, though it was agreed this was only possible during the summer season in southern Canada.
- The average lower temperature tolerance of White Tetra was reported in the notification to be 7.84°C when temperature is dropped rapidly (0.5 to 2°C/hour). When temperature was dropped gradually (1°C/day), Leggatt et al. (2017) observed that White Tetras decreased feeding and activity at 17°C, stopped feeding at 12°C, and lost homeostasis at 9.95°C. The notifier reported 7.98°C as the average lethal temperature to CGT2016 when temperature is dropped rapidly.
- It was clarified that the notifier contracts production of CGT2016 to two companies in Florida.
- This Risk Assessment covers only the CGT2016; further notifications will be necessary if the notifier wants to market the other colours as they represent different genetic modifications.

KEY SUMMARY POINTS OF THE DISCUSSION

- The nature of the transgene construct is unlikely to cause any harm to the environment or indirect human health;
- The nature of the transgene insert at the locus, while unconfirmed, is unlikely to cause harm to the environment or to indirect to human health;
- Mendelian inheritance (from crosses using only 10 pairs of fish) and molecular stability of the locus has not been adequately demonstrated;

- It is highly probable that aquarium enthusiasts in Canada will breed CGT2016; and
- Low temperature tolerance limits of the comparator species and CGT2016 is likely to be the largest factor limiting survival.

CHARACTERIZATION OF THE RECEIVING ENVIRONMENT

Presenter: Colin McGowan, Fisheries and Oceans Canada

The "Characterization of the Receiving Environment" presentation examined Canadian bodies of freshwater that could receive CGT2016, with a focus on temperature. CGT2016 is a tropical fish, its ability to survive year-round in the Canadian freshwater environment is expected to be limited by cold intolerance. CGT2016 is a freshwater fish and would likely be restricted to bodies of freshwater; such as any freshwater spring, stream, pond, river, lake, or reservoir. It was emphasized that water temperature is a key abiotic factor that affects both the survival and reproduction of most freshwater fish populations, and is a pervasive determinant of habitat suitability.

In some of the warmer lakes in Canada, water temperatures may range between 20 and 26°C for parts of the year, and can remain in the optimal range for tropical fish for extended periods during the summer months. However, minimum water temperatures reach near 0°C, and are generally lower than 5°C during the winter months. There are only two or three surveyed lakes in Canada where temperature may remain at 6°C or above during the winter. In all other lakes, seasonal turnover homogenizes the temperature of the water column when heavy surface water at 4°C sinks to the bottom, mixing the stratified layers to a uniform 4°C. Almost all lakes in Canada undergo lake turnover at least once per year. Consequently, if an introduced fish cannot survive at 4°C or below, its occurrence in the Canadian environment will be seasonal at best, with possible locations to overwinter, if the organism can survive temperatures of 6°C or lower.

DISCUSSION

Participants had concerns about the completeness of seasonal turnover, and discussed if localized areas may not be subject to temperature homogenization, allowing for the survival of CGT2016. The wild-type tetra prefers habitat in shallow, slow moving water with vegetation, and is most likely to stay near the surface or near the shoreline of water bodies. Such locations are expected to freeze regardless of turnover.

Other potential sources of micro-heterogeneity in water bodies within which the CGT2016 could survive throughout the year, such as warm pockets of sewage or industrial effluent and hot springs were discussed.

There was some concern over the possibility and consequences of interspecies hybridization between CGT2016 and wild endemic/local fish or with wild-type tetra. Though inter-species hybridization has not been empirically tested, it is unlikely to occur as Tetra species do not occur in Canada.

There was discussion that climate change scenarios should be addressed in the uncertainty ratings, rather than the exposure ratings and should include more content on seasonality and effects during winter. In particular, shallow freshwater coastal environments should be a focus of this particular characterization because tetras are expected to be found in the shallow freshwater shorelines, rather than the middle of deep lakes or swift running streams.

KEY SUMMARY POINTS OF THE DISCUSSION

- It was emphasized that shallow freshwater shoreline should be a focus of this particular characterization.
- It was suggested that some text should be added to the risk assessment about climate change and potential to affect survivability of CGT2016 in the future; with regard to shallow freshwater shorelines in particular.
- Overwinter survival likelihood in Canada is extremely small.

INDIRECT HUMAN HEALTH RISK ASSESSMENT

INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT

Presenter: Kassim Ali, Health Canada

The "Indirect Human Health Risk Exposure Assessment" presentation addressed the exposure and associated uncertainties regarding the exposure of CGT2016 to humans through the environment. The IHH exposure assessment process involved identifying the sources of exposure, the individuals likely to be exposed (healthy, immunocompromised, children, those with underlying medical conditions), and potential routes of exposure. Oral ingestion is considered a food safety aspect and not addressed under CEPA assessment. The assessment is accomplished through estimation of the level of the identified exposure (quantitatively or qualitatively), and the trends in exposure levels over time. Likelihood of exposure is characterized in terms of the likelihood of human exposure, which is rated low, medium, or high and described as:

- **Low**, if there is intended use (no intentional release), if the nature of release and/or the biology of the organism are expected to contain the organism such that susceptible populations or ecosystems are not exposed, or if low quantity, duration and frequency of release of organisms that are not expected to survive, persist, disperse or proliferate in the environment where released;
- **Medium**, if the organism is released into the environment, but quantity, duration and/or frequency of release is moderate, or if the organism persists in the environment in low numbers, if the potential for dispersal/transport is limited, if the nature of release is such that some susceptible living organisms may be exposed, and finally if routes of exposure are not expected to favour toxic, zoonotic or other adverse effects;
- **High**, if the release quantity, duration, and/or frequency are high, if the organism is likely to survive, persist, disperse, proliferate, and become established in the environment, or if dispersal or transport to other environmental compartments is likely, if the nature of release makes it likely that susceptible living organisms or ecosystems will be exposed and/or that releases will extend beyond a region or single ecosystem, and finally if in relation to exposed organisms, routes of exposure are permissive of toxic, zoonotic or other adverse effects in susceptible organisms.

Uncertainty associated with the assessment on exposure to the Canadian environment was ranked as follows:

- **Negligible**, if there are high quality data on the organism, the sources of human exposure, and the factors influencing human exposure to the organism, and if there is evidence of low variability;

- **Low**, if there is high quality data on relatives of the organism or valid surrogates, if there is high quality data on the sources of human exposure and the factors influencing human exposure, and if there is evidence of variability;
- **Moderate**, if there is limited data on the organism, relatives of the organism, or valid surrogates, and if there is limited data on the sources of human exposure and the factors influencing human exposure to the organism;
- **High**, if there are significant knowledge gaps and significant reliance on expert opinion.

Based on data available from the notifier regarding the projected number of Canadian outlets to import CGT2016, the exposure during import is expected to be low. Similarly, based on the number of Canadian households estimated to purchase CGT2016, exposure during introduction is expected to be low. These estimates were based on the intended use of CGT2016, which involves retail outlets and home aquariums. The non-intended uses of CGT2016, identified as including intentional release to the environment and use as bait fish, were determined to be minor because no additional risks were foreseen that were different from any other aquarium fish.

The environmental fate of the tropical fish CGT2016 following deliberate or unintentional environmental release was suggested to be curtailed by limited cold tolerance. CGT2016 cannot tolerate cold temperatures (< 8°C), there is no history of invasiveness, and it was assumed that fluorescent green colouration may lead to increased predation. Therefore, CGT2016 pose no greater risk of bioaccumulation or involvement in biogeochemical cycling than other released aquarium fish.

Exposure was characterized as fish being confined (not contained) in home aquariums and retail outlets, with potential for 'some' individuals to be exposed. Typical human exposure is expected to occur during maintenance activities such as tank cleanings and water changes.

Consequently, the IHH exposure was suggested to be low to medium with moderate uncertainty.

Discussion

Discussions after the presentation covered a variety of issues.

The assumption that predation would be higher for CGT2016 than for the wild-type due to its bright colouration was an issue of concern because of limited and conflicting empirical evidence. Only three published studies exist, the results of which demonstrate that predation on GE fluorescent fish can be lower, higher, or the same as for the wild-type colouration. Discussion ensued on whether the potential for released CGT2016 to be consumed as prey should be mentioned. Because of their small size, they are expected to be a potential prey item for several native predators in Canada, which may limit their persistence and hence exposure through the environment.

With regard to environmental fate and other uses, it was clarified that the possibility of release is very high. Exposure is likely to be highest from cleaning fish tanks, rather than a release into the environment. Though household use must be addressed, it remains that there is still a high possibility of release to the environment. Depending on how these pathways are weighted, a rank for exposure could be either low (through environmental release) or medium (within households) at the scale of the overall Canadian population.

The different avenues taken by HC and DFO to arrive at an exposure rating was discussed. HC uses mid-categories (e.g., low-medium), whereas DFO picks the highest of two possible categories.

The following changes to the indirect human health assessment were recommended:

- Remove the statement on increased predation from the risk assessment.
- Change the statement on low likelihood of release to high, and clarify that this is considered a full release scenario. The perspective in the "Exposure Characterization" section needs to reflect the full release scenario.
- Modify the wording to reflect clearly that the environment of the aquarium is what is being referred to versus release to nature at any given part in the text on exposure through intended use.

Consensus

Participants reached consensus on the following:

- The assessment concludes with moderate uncertainty that the potential for CGT2016 exposure to the Canadian public is low to medium.
- There remains the option to re-open discussion on changing the exposure rating, should more information be put forward or uncovered during the meeting.

INDIRECT HUMAN HEALTH HAZARD ASSESSMENT

Presenter: Stephen Dugan, Health Canada

The "Indirect Human Health Risk Assessment" presentation addressed the capacity of CGT2016 to act as a vector for human pathogens, as well as its toxicity, allergenicity, and general health status. The IHH risk assessment only considered hazards that could result from environmental exposure to CGT2016, through activities such as the cleaning of an aquarium. It did not include potential hazards associated with consumption of CGT2016 as food (considered under the *Food and Drugs Act*), or occupational health hazards (considered under the *Occupational Health and Safety Act*). A comparative (incremental) hazard assessment approach was taken to determine the potential of CGT2016 to act as a vector for pathogens, and its potential toxicity and allergenicity, relative to White Tetra.

Hazard rankings used for the indirect human health risk assessment were described as follows:

- **Low**, if no effects on human health or effects are expected to be mild, asymptomatic, or benign in healthy individuals, there are effective prophylactic treatments available, and if there is no potential for community level effects;
- **Medium**, if effects on human health are expected to be moderate but rapidly self-resolving in healthy individuals and/or effective prophylactic treatments are available, and if there is some potential for community level effects;
- **High**, if effects in healthy humans are severe, of longer duration and/or sequelae in healthy individuals or may be lethal, and there are no prophylactic treatments available or they are of limited benefit, and if there is high potential for community level effects.

Uncertainty associated with each hazard was ranked as follows:

- **Negligible**, when there are many reports of human health effects related to the hazard and the nature and severity of the reported effects are consistent (i.e., low variability), or when the potential for human health effects in individuals exposed to the organism has been monitored and there are no reports of effects;

- **Low**, when there are some reports of human health effects related to the hazard and the nature and severity of the effects are fairly consistent, or when there are no reports of human health effects and there are no effects related to the hazard reported for other mammals;
- **Moderate**, when there are some reports of human health effects that may be related to the hazard but the nature and severity of the effects are inconsistent, or when there are reports of effects related to the hazard in other mammals but not in humans.
- **High**, when there are significant knowledge gaps (e.g., there have been a few reports of effects in individuals exposed to the organism but the effects have not been attributed to the organism).

The potential for CGT2016 to act as a vector for human pathogens was reviewed. Bacterial species associated with zoonoses from ornamental fish include *Aeromonas* sp., *Mycobacterium marinum*, *Salmonella* sp., and *Streptococcus iniae*; all of which can be transmitted to humans through activities such as cleaning aquaria and handling fish. Cases of severe zoonoses (e.g., tuberculosis) are very rare. Infections are expected to occur primarily from secondary infections in skin wounds, such as cuts, scrapes, and abrasions. Preventative measures include wearing gloves and washing hands with soap and water. Though parasitic fish-borne zoonoses may result from consumption of raw or improperly cooked fish, there have been no reported zoonoses attributed to CGT2016 or *G. ternetzi*.

The human hazard potential of CGT2016 was assessed to be low. The methods used to produce the notified living organism do not raise any human health concerns, and the sources of the inserted genetic material are not pathogenic. While there are reported cases of zoonotic infections associated with tropical aquarium fish, there are no reported cases attributed to either the notified strain or the wild-type. The sequence identity of the inserted transgene does not match any known allergens or toxins. Finally, there is a history of safe use for the notified line in the United States and for the wild-type species as an ornamental aquarium fish with no reported adverse human health effects in the literature. The level of uncertainty associated with this conclusion is low, since there was sufficient information based on reports from other species of ornamental fish as well as a history of safe use, and thus contributing to some uncertainty in the hazard conclusion. Consequently, the indirect human health hazard assessment of *G. ternetzi* CGT2016 is concluded be low, with low uncertainty.

Discussion

After the presentation, questions regarding the rating levels were raised. DFO used a four level system (negligible, low, moderate, high), whereas HC used a three level system (low, medium, high) to rate hazard. It was clarified that uncertainty was rated as low, and not negligible, due to a lack of direct studies. Participants discussed the issue of non-proposed uses. Several non-proposed uses were postulated, such as use as bait fish, environmental indicators, or rearing in outdoor ponds; however, the assessment may not have identified all possible non-proposed uses. Ingestion by humans would be considered a non-proposed use, and would therefore be considered a 'direct' human health concern, not indirect. Finally, it was clarified that this indirect human health assessment takes into account reports that could include people with pre-existing conditions, or do not practice preventative measures.

Consensus

Participants reached consensus on the following:

- A statement explaining that CGT2016 transgenic fish cause no more health hazards than the wild-type (comparator) species be added to the text of the IHH hazard risk assessment.
- The assessment concluded with low uncertainty that the potential indirect human health hazard associated with CGT2016 toxicity, allergenicity, and novel or endogenous toxins is low.

INDIRECT HUMAN HEALTH RISK ASSESSMENT

Presenter: Kassim Ali, Health Canada

The "Indirect Human Health Risk Assessment" presentation addressed the exposure to indirect human health hazard outcomes, and concluded on the indirect human health risk. The indirect human health exposure and hazard characterizations from previous talks during the meeting were summarized, followed by a two-part view of the overall risk characterization, first, based on the notified use (as an aquarium fish for hobbyists), and second, based on other potential uses (released to outdoor ponds, as a bait fish, in scientific research, or as an environmental sentinel).

Based on the notified use, the indirect human health risk potential of CGT2016 was assessed to be low because:

1. *G. ternetzi* CGT2016 is a genetically modified green-coloured tropical fish containing a single copy of a transgenic construct, and that was derived from a naturally occurring white variant of the Black Tetra;
2. Sequence identity of the inserted transgene does not match any known allergens or toxins;
3. CGT2016 will be marketed throughout Canada as an ornamental fish in home aquariums where exposure is mainly through cleaning and maintenance activities;
4. While there are reported cases of zoonotic infections associated with tropical aquarium fish, the Black Tetra is a popular aquarium fish with a long history of safe use and with no reported cases in the literature;
5. Owing to the low potential hazard and the low to medium potential exposure, the human health risk associated with the use of CGT2016 for use as an ornamental aquarium fish is assessed to be low.

Other potential uses include and are assessed for indirect human health risk as follows:

1. Since CGT2016 is considered attractive to predators, it may be used as a bait fish. It can be grown in outdoor ponds when temperatures are favourable. With the published patent, its use as a model research organism is also possible;
2. There are no reported cases in the literature of CGT2016 being used as an environmental sentinel and CGT2016 would not be an appropriate or useful model for an environmental sentinel given the nature of the transgene construct;
3. Available information provides no indication of potential human health impacts from any of these uses.

The presentation concluded that there is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from use of CGT2016 as an ornamental aquarium fish or other potential unintended uses.

Therefore, the risk to human health associated with CGT2016 is considered to be low and is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

Discussion

It was clarified that low risk was a term that is applied to the general population, and that there are uncertainties that could lead to a change in the conclusion of low risk. It was suggested by participants that this should be captured in the uncertainty section of the Science Advisory Report.

Consensus

The overall indirect human health risk associated with the import, introduction, and notified use of CGT2016 was concluded to be low. The overall uncertainty level and any caveats to the final conclusions are addressed in the Science Advisory Report.

ENVIRONMENTAL RISK ASSESSMENT

ENVIRONMENTAL EXPOSURE ASSESSMENT

Presenter: Colin McGowan, Fisheries and Oceans Canada

The "Environmental Exposure Assessment" presentation provided an overview of the presumed stages of events from release to spread of CGT2016 in the natural environment: release, survival, persistence, reproduction, proliferation, and geographic spread. The likelihood of each of these steps/stages was considered in forming a conclusion regarding the potential fate of CGT2016 in the environment, if released. The presentation also delineated the rankings used for the likelihood of exposure and associated uncertainties.

Likelihood of exposure ranking used for the environmental risk assessment was described as the following:

- **Negligible likelihood**, if there is no occurrence in the environment and the comparator species has not been observed in the Canadian environment;
- **Low likelihood**, if there are instances of rare, isolated occurrences with an ephemeral presence;
- **Moderate likelihood**, if exposure often occurs, but only at certain times of the year or in isolated areas;
- **High likelihood**, if exposure often occurs at all times of the year and/or in diffuse areas.

Uncertainty associated with the assessment on exposure to the Canadian environment was ranked as follows:

- **Negligible**, if there is high quality data on the organism (e.g., sterility, temperature tolerance, fitness), the data on environmental parameters of the receiving environment and at the point of entry, if absence of genotype-environment interaction (GxE) effects have been demonstrated or if there is complete understanding of GxE effects across relevant environmental conditions, and if there is evidence of low variability;
- **Low**, if there is high quality data on relatives of the organism or valid surrogates, data on environmental parameters of the receiving environment, and/or an understanding of potential GxE effects across relevant environmental conditions, as well as evidence of variability;
- **Moderate**, if there is limited data on the organism, relatives of the organism or valid surrogate. Limited data on environmental parameters in the receiving environment.

Knowledge gaps. Reliance on history of use or experience with populations in other geographical areas with similar or better environmental conditions than in Canada.

- **High**, if there are significant knowledge gaps and significant reliance on expert opinion.

There is a high likelihood that CGT2016 will be introduced to Canadian environment. The practice of releasing aquarium fish into the environment is common and ongoing. Since there is no true control over CGT2016 once it has been sold, it is appropriate to consider it under a full-release scenario.

Water temperature is a key abiotic factor that affects both the survival and persistence of most freshwater fish populations. Experiments conducted independently by DFO and GloFish LLC suggest CGT2016 cannot survive at temperatures below 7°C, and likely cannot survive extended periods at temperatures below 9.5°C. Though water temperatures needed for CGT2016 to survive are possible for several Canadian lakes during spring, summer and fall; no lakes in Canada remain above 6°C throughout the entire course of the year. Therefore, occurrence of CGT2016 would be seasonal or ephemeral at best.

Though water temperature is expected to limit occurrence and lifespan of CGT2016, there is still a potential for reproduction. Any opportunities for reproduction are expected to be isolated and short lived, and could not surpass a single generation.

The capacity for CGT2016 to proliferate and spread in the Canadian environment is precluded by the fact that White Tetras cannot survive the Canadian winter. A possible exception would be within a minimal number of isolated pockets of warm water (e.g., hot springs, warm water industrial effluent). However, previous release of Black Tetras to a Colorado hot spring failed to establish. Anecdotal evidence from the ornamental aquarium trade indicates Black Tetra have very specific temperature requirements for successful reproduction. Consequently, the potential for White Tetras and CGT2016 to establish isolated populations in thermal pockets of freshwater in Canada is unlikely.

Overall, it was concluded with negligible uncertainty that the likelihood of CGT2016 exposure to the Canadian environment is low.

Discussion

Discussion following the presentation clarified the expected annual rate of releases. Previous studies report approximately 2% of unwanted aquarium fish are released to the wild, based on a survey of Ontario aquarium owners (Marson et al. 2009), while a study in the US suggested approximately 2,500 aquarium fish are likely released into Puget Sound, Washington on an annual basis (Strecker et al. 2011).

A discussion was held on genotype x environment (GxE) interaction effects on cold tolerance. Living in variable environment can allow for variability in expression of phenotype. However, the bounds of cold tolerance relative to Canadian winter temperatures are so restrictive that they render the range for survival too narrow to be an issue with this particular species. Participants agreed that most CGT2016 would not survive the temperature limitations, regardless of the breath of phenotypic plasticity over a single, as well as a few generations. Therefore, long-term survival of the CGT2016 in Canadian environment is very unlikely.

A question arose regarding the temperature tolerance curves (survival versus temperature), and whether temperature tolerance was a heritable condition. Inheritance of variability has not been studied for any trait in Black Tetra, though variability in cold tolerance for other species has been studied (although less so than for high temperature tolerance), including escaped tropical ornamental fish.

Some meeting participants recommended that the negligible uncertainty rating be changed to low, due to lack of direct studies and data on CGT2016 (as noted above).

Consensus

Participants reached consensus on the following:

- The assessment concludes that the environmental exposure level is low.
- The quality of temperature tolerance data makes the uncertainty associated with the conclusion on exposure to be low.

ENVIRONMENTAL HAZARD ASSESSMENT

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The "Environmental Hazard Assessment" presentation examined the potential for CGT2016 to cause harmful effects to the environment due to the targeted phenotype or off-target effects. It considered potential hazards to environmental components (e.g., biodiversity), and ranked hazards depending on the presence and reversibility of harmful effects.

Hazard ranking used for the environmental hazard assessment were described as:

- **Negligible**, if there are no effects;
- **Low**, if there are no harmful effects;
- **Moderate**, if there are reversible harmful effects; or
- **High**, if there are irreversible harmful effects.

The uncertainty characterization was preceded by a brief description of the knowledge gaps and lack of empirical data associated with CGT2016 environmental hazards. Data for CGT2016 or comparator species in natural environments were not available, thus the hazard assessment relies solely on laboratory data. Additionally, it was noted that the effects of genotype, environment, and GxE interactions when relying on laboratory data can contribute to uncertainty. Ultimately, the quality of data dictates the level of uncertainty that was ranked as:

- **Negligible**, if there is high quality data, absence of GxE or complete understanding of GxE, and evidence of low variability;
- **Low**, if there is quality data on relatives of CGT2016 or valid surrogates, an understanding of GxE effects, and only some variability;
- **Moderate**, if there are limited data on CGT2016, as well as on the relatives of CGT2016 or valid surrogate, there is a limited understanding of GxE effects, there are knowledge gaps, and there is a reliance on expert opinion rather than empirical data; or
- **High**, if there are knowledge gaps and a reliance on expert opinion.

Eight different hazard endpoints were assessed:

1. Potential environmental toxicity
2. Potential for hazards via gene transfer
   a. through hybridization
   b. through horizontal gene transfer
3. Potential impacts through interactions with other organisms

4. Potential to act as a vector of disease agents
5. Potential to impact biogeochemical cycling
6. Potential to affect habitat
7. Potential to affect biodiversity

1. Potential environmental toxicity

Hazard considerations regarding the potential environmental toxicity of CGT2016 were reviewed. Fluorescent proteins naturally occur in many marine species, and are commonly used as neutral-marker transgenes in many research animals. Fluorescent transgenes have been used in ornamental fish species in the US since 2003. CGT2016 has been in commercial use since 2012. Based on the molecular characterization of the transgene construct, lack of toxic effects to rats, and no matches to known allergens, it was concluded that CGT2016 posed negligible potential for environmental toxicity with negligible uncertainty associated with this rating.

Discussion

Use of the word "toxicity" during the presentation was clarified to indicate that the protein itself was, or was not, toxic to other organisms and was not being used in reference to "CEPA toxic".

Concern was raised regarding the negligible uncertainty rating. It was decided that since the assessment was based on data available for the green fluorescent protein (GFP), a valid surrogate, rather than direct evidence based on the notified organism or its specific fluorescent protein, the uncertainty rating should be higher than negligible.

Regarding a reference to Allermatch, it was clarified that the database applies more to humans than to wild organisms, and that we do not have a good understanding of the effects of the protein on other fish.

The wording of the Hazard rating table was discussed in this section and the following section, particularly regarding the negligible and low categories and the scope of each rating (effects on individual versus effect on structure and function of the ecosystem). The meeting adjourned with resolution to re-write the table in the morning as a group, before going through the rest of the hazards. As a consequence, the hazard rankings used for the environmental hazard assessment were changed to include clarifications to the negligible and low hazard rating as follows:

- **Negligible**, if there are no effects (i.e., No biological response expected beyond natural range);
- **Low**, if there are no harmful effects[1].

[1]*Harmful effect: an immediate or long-term detrimental impact on the structure or function of the ecosystem including biological diversity beyond natural fluctuations.*

Participants reached consensus that the scope of the assessment was at the population and ecosystem level. For example, an organism might cause an allergic reaction in one individual, but if that reaction does not change the structure or function of the ecosystem, then it can be considered as no harmful effect.

The evaluation of hazard ratings ensued by going back through the first two endpoints on toxicity and gene transfer. The text of the proceedings that follows considers discussion and consensus based on the updated hazard rating wording.

Consensus

- The assessment concluded with moderate uncertainty that CGT2017 represented a negligible toxicological hazard to populations or the structure and function of the ecosystem.

2. Potential for Hazards via Gene Transfer

Consideration was given to the potential for gene transfer of the transgene through both hybridization and horizontal gene transfer (HGT).

2.a) Gene transfer through hybridization

A negligible hazard rating with negligible uncertainty was proposed as there are no species of the same taxonomic family as *G. ternetzi* endemic to Canada, and interbreeding of CGT2016 with endemic fish is not biologically possible. Therefore, CGT2016 is not expected to hybridize and no effects via hybridization are expected.

Discussion

It was clarified that the rating concerning hybridization should be made on the environmental 'effects' of gene transfer, rather than occurrence of gene transfer. Concern was raised regarding the lack of direct evidence on CGT2016 interbreeding behaviour, which would lead to a low hazard rating. After some discussion, all agreed that the weight of evidence relies on a lack of the entire Family of this tropical fish in Canadian waters. Thus, overall there is ample evidence with which to make a decision, and the hazard should remain as negligible.

There was consensus that a statement must be made in the text explaining that Black Tetra are broadcast spawners, and as such there are no behavioural impediments to hybridization, although any accidental hybridization with native species that also broadcast spawn are not expected to be viable.

Consensus

- The assessment concluded with negligible uncertainty that CGT2017 represented a negligible potential for hazards via gene transfer through hybridization with other fish.

2.b) Considering gene transfer through horizontal gene transfer (HGT)

Horizontal gene transfer (HGT) requires 1) exposure/uptake of the free transgene to a novel organism, 2) stability and expression of the gene within the novel organism, and 3) neutral or positive selection of the novel organism expressing the transferred gene. Exposure of free transgenic DNA to susceptible species is expected to be low. While location of transgene relative to transposable elements is not known, potential uptake of the transgene beyond wild-type genes is negligible due to lack of mobile elements within the transgene construct. Therefore, potential gene transfer via HGT is not expected to cause harmful effects but uncertainty level is not negligible.

Discussion

There was a lengthy discussions on HGT. Overall, it was clarified that when considering HGT, whether gene transfer 'could' occur was not an effect beyond natural fluctuation (i.e., it is a common prokaryotic mechanism), but whether HGT then resulted in an effect beyond natural fluctuations, and whether that effect was harmful needed to be assessed. Although it is theoretically possible for HGT of the transgene to occur, the probability of such an occurrence is low. For horizontal gene transfer to cause a biological effect at the population level, the addition of the new gene has to be selectively advantageous and confer a fitness benefit. Bacteria are

highly selective of the genes they uptake, due to associated costs. Therefore, uptake does not happen readily at the population level for any new gene encountered. Although non-related fluorescent proteins have been identified in some Canadian freshwater bodies, should HGT of the construct occur it would introduce a new marine-origin gene into the environment, which is considered beyond the natural range. However, the transgene does not result in significant harmful effects to CGT2016, and is consequently not expected to cause harmful effects to prokaryotic hosts or their environment. It was agreed that CGT2016 release may potentially create a new strain of fluorescent bacteria with the transgene, and the hazard rating should remain as low, and not be changed to negligible.

Consensus

- The assessment concluded with low uncertainty that CGT2016 represented a low potential for hazards via gene transfer through horizontal gene transfer to non-eukaryote species.

3. Potential impacts through interactions with other organisms

Hazard considerations regarding the potential impacts of CGT2016 through interactions with other organisms (hybridization, predation, competition) were reviewed. As stated above, there is a negligible impact potential with negligible uncertainty through hybridization with endemic or non-native fish. However, CGT2016 may impact native organisms through trophic interactions as a competitor, predator or prey.

The Black Tetra is not known to be highly competitive or aggressive in aquarium settings, but in the wild may compete with other small carnivorous fish or insects. However, decreased activity/feeding below 17°C will limit their ability to compete, act as predator, or avoid predation in Canadian waters throughout most of the year. In 5 years of use of CGT2016 in the US, there are no reports of altered behaviour that may impact trophic interactions.

It was suggested that the bright colouration of CGT2016 may increase the likelihood of predation on the organism in the wild. One study in India reported RFP Zebrafish survived well relative to wild species under laboratory conditions (Jha 2010), whereas other studies report RFP Zebrafish are equally or more preyed upon than wild-type (Cortemeglia and Beitinger 2006; Hill et al. 2011). Inconsistencies among these studies may be the result of genetic background, strain effects, rearing history, experimental conditions, or GxE effects. The relevance of RFP Zebrafish studies to the biology CGT2016 is unknown.

Overall, the hazard rating for potential impacts through interactions with other organisms was determined to be negligible, due to an absence of highly competitive/aggressive behaviour in Black Tetra, decreased activity of Tetra in low temperatures, and the apparent absence of behavioural changes resulting from the transgene. The lack of studies directly examining CGT2016 and limited understanding of GxE interactions resulted in an uncertainty ranking of moderate.

Discussion

Concern was expressed that the negligible hazard ranking should be changed to low because species interactions will undoubtedly occur. However, support for the negligible rating was given with the rationale that the impact would be no larger than from any other fish present (i.e., within normal distribution). Several examples of how flawed studies can sway uncertainty ratings were discussed.

It was stated that the exposure level of CGT2016 in the wild could be extreme, as aquarium enthusiasts could conceivably breed and release thousands of fish. In that situation, a greater impact may be realised when compared to small scale and isolated releases. Regardless, the

panel agreed that the hazard to other organisms would remain negligible under this scenario with all participants in agreement that we are considering hazard in the context of populations, and the structure and function of the ecosystem.

It was clarified that the term "beyond natural range" refers directly to the phenotype resulting from the gene modification. When questioned if the gene modification increased the hazard potential at a population or ecosystem level, group consensus was that CGT2016 posed no greater hazard than wild-type fish. It was also agreed that the environmental hazard assessments should be viewed in comparison with base species to help reach conclusions (as was done with the IHH assessment).

Consensus

Participants reached consensus on the following conclusions:

- The hazard rating for potential impacts of CGT2016 through interactions with other organisms was concluded to be negligible, with moderate uncertainty due to the high variability in the available published data.
- All participants agreed that new wording, "beyond natural range", added to the hazard ratings, prohibits the trap of assessing 'individual effects' by considering hazard at the population level. Ultimately the new wording helps to distinguish between the rankings of negligible and low.

4. Potential to act as a vector of disease agents

Any disease agents CGT2016 may be carrying are expected to persist in heated waters (e.g., 25-28°C) normally found in home aquaria, and may have limited persistence in Canada's temperate to arctic climates. The Black Tetra is not listed by CFIA as carrying disease agents of concern to Canada.

The disease vector capability of CGT2016, or of any other fluorescent organism, has not been examined. Veterinarian statements provided by GloFish LLC stated no noted increased susceptibility to, or transmission of, water-borne pathogens, or additional health impediments of CGT2016 or any other fluorescent line produced by GloFish. Howard et al. (2015) tracked wild-type and RFP Zebrafish over 15 generations and reported no differences in survival between the two groups.

Overall, the available information indicates no expected detrimental effects above that of wild-type Black Tetra. Consequently, CGT2016 poses a negligible hazard as a vector of disease as a result of the genetic modification. A moderate uncertainty rating was proposed since CGT2016 itself has not been directly examined for vector capabilities, and there is reliance on indirect evidence and expert opinion.

Discussion

The brief discussion began with comments and concerns regarding the new genes and the physiological trade-offs associated with the total proportion of CGT2016's metabolism devoted to maintenance compared to non-genetically modified Black Tetra. The CGT2016 may be more likely to carry pathogens, because they could possibly be metabolically compromised by the need for production of the new proteins. If CGT2016 is more susceptible to disease, it could either introduce more disease agents into the environment, or, could die off more quickly.

It was clarified that the Black Tetra has never been found to carry disease of identified economic or environmental importance to Canada, though GloFish veterinarian statements identified gill parasites on CGT2016, which are common in ornamental aquarium fish. Since there is no

evidence of greater incidence of disease or vector capabilities in CGT2016 relative to Black Tetra, a negligible hazard rating was agreed upon.

Consensus

- The hazard rating on potential of CGT2016 to act as a vector of disease was concluded to be negligible with moderate uncertainty, due to the lack of evidence on vector capabilities of CGT2016 and reliance on expert opinion.

5. Potential to impact biogeochemical cycling

CGT2016 is expected to contribute to nutrient cycles through ingestion of prey and release of metabolic waste. The Black Tetra is described as 'not over-eating', thus causing limited waste in the aquarium setting. The lack of excessive metabolic waste combined with their small size means their capacity to impact nutrient cycling is limited. It should be noted, however, that there are no data on the effects of the construct's fluorescent protein on metabolism and nutrient cycling. A single report on enhanced GFP (eGFP) transgenic mice found that their urea cycle and metabolism were altered compared to wild-type mice, but, whether the construct impacts nutrient cycling or alters CGT2016 metabolism is not known. The small size of CGT2016 suggests that impacts to biogeochemical cycling would be minimal even if transgene expression alters CGT2016 metabolism. Consequently, there are no expected detrimental effects to biogeochemical cycling from CGT2016. Based on the small size of CGT2016 and lack of polluting characteristics in Black Tetra, a negligible hazard rating related to the potential to impact biogeochemical cycling was proposed with moderate uncertainty, due to the lack of studies on CGT2016 and biogeochemical cycling.

Discussion

All participants were in agreement with the suggested negligible hazard with moderate uncertainty. The potential for urea cycling enzymes to alter gene expression levels was raised, but was not discussed at length.

Consensus

- The hazard rating on potential of CGT2016 to cause harmful effects to biogeochemical cycling was concluded to be negligible with moderate uncertainty, due to the lack of direct evidence on CGT2016.

6. Potential to impact habitat

The Black Tetra is a small fish with negligible potential to impact habitat structure. They spawn in open water and do not build nests or other structures that may impact the habitats of other species. There are no reports of CGT2016 having an altered behaviour or phenotype that may influence natural habitat structure. As there is no evidence of effect to habitat of Black Tetra and no reports of alterations in CGT2016 that may influence habitat, a negligible hazard to habitat was proposed with negligible uncertainty.

Discussion

There was discussion of how a surrogate organism, Black Tetra, provides good information and data, given it has a history of 70 years of husbandry. However, it was argued that 'in the tank' information is still merely anecdotal and not scientific evidence of effects in nature. On the basis of the latter sentiment, the low uncertainty ranking was suggested, due to a lack of direct observations of the surrogate organism in multiple environments.

Consensus

- There is no evidence of effects to habitat by Black Tetra.
- The hazard ranking on potential of CGT2016 to impact habitat is negligible with low uncertainty.

7. Potential to affect biodiversity

There are no reports of Black Tetra listed as an invasive species worldwide, despite many decades of use. Decreased reproductive success and decreased cold tolerance may decrease invasiveness potential, and there is no evidence of increased fitness in CGT2016 that may increase invasiveness. CGT2016 are not expected to impact biodiversity through disease transmission, toxicity, interactions with native species, or through impacts to biogeochemical cycling and habitat. Consequently, it was proposed with low uncertainty that CGT2016 pose a negligible hazard to biodiversity of Canadian ecosystems.

Discussion

The question of whether or not CGT2016 could displace existing populations or create new populations in Canada was raised. It was generally agreed that CGT2016 could not displace or create new populations, due to lack of aggression, small size, lack of cold tolerance, and various other characteristics discussed in addition to a large body of surrogate organism data.

Consensus

- The hazard rating on potential of CGT2016 to affect biodiversity was concluded to be **negligible** with **low uncertainty**.

OVERALL SUMMARY DISCUSSION AND CONSENSUS ON ENVIRONMENTAL HAZARDS

A discussion was undertaken to summarize the environmental hazard potential of CGT2016, with the main task to aggregate the eight final hazard and uncertainty ratings into a single overall rating.

The first suggestion was to rate the hazard as low with an overall moderate rating for uncertainty. It was pointed out that the overall rating is not a true reflection of each hazard category; however this variation will be reflected in the overall risk assessment.

It was suggested that the gene transfer hazard section be divided into two separate parts A) gene transfer through hybridization and B) horizontal gene transfer to other organisms, namely prokaryotes. The discussion went back to HGT at length. Ultimately, due to the possibility that CGT2016 releases might potentially create a new population, or affect the evolutionary trajectory of populations of bacteria, it was agreed that the hazard rating remains low, and should not be changed to negligible.

It was stressed that any Hazard associated with "unknown" consequences must be made clear in the environmental risk assessment and in place of a simple statement on the fact that HGT will occur, it should be emphasized that that is an occurrence we cannot discount, but do not expect to be harmful.

It was stated that it is critical to clarify in the text which ratings might change in the event of new research (e.g., biogeochemical cycling, disease).

It was agreed among meeting participants that uncertainty associated with hazard and exposure ratings, in particular, must be articulated to regulators. The rationale behind this conclusion and

discussion was that each hazard has a role and should be considered separately. The rating itself may be considered transient, because uncertainty levels are high enough to warrant future studies in these areas.

After the summary discussion, participants reached consensus on the following conclusions:

- The assessment concludes with moderate uncertainty that CGT2016 represents a negligible hazard through environmental toxicity.
- The assessment concludes with low uncertainty that the potential hazard that may result from horizontal gene transfer from CGT2016 is low, because surrogate models show ample evidence of no harmful effects. Although it is theoretically possible for horizontal gene transfer to occur to freshwater prokaryotes (e.g., bacteria), the probability appears to be low.
- The assessment concludes with negligible uncertainty that the potential hazard that may result from gene transfer through hybridization of CGT2016 with other fish is negligible.
- The assessment concludes that the potential hazard through interactions with other animals of variation is negligible (considering that effects from CGT2016 interactions are not expected to deviate from those found in nature) with moderate uncertainty (due to the lack of consistent results in the available published data on surrogate organisms).
- The assessment concludes that the potential hazards as a vector of disease agents is negligible with moderate uncertainty. Based on the evidence presented, there is no greater level of risk from CGT2016 versus Black Tetra.
- The assessment concludes with moderate uncertainty that the potential to impact biogeochemical cycling is negligible.
- The assessment concludes with low uncertainty that the potential of CGT2016 to impact habitat is negligible.
- The assessment concludes with low uncertainty that the potential of CGT2016 to affect biodiversity is negligible.

ENVIRONMENTAL RISK ASSESSMENT

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The "Environmental Risk Assessment" presentation addressed the exposure outcome, the environmental hazard outcomes, and concluded on the environmental risk.

The risk assessment was based on the classic risk paradigm where:

Risk = Exposure x Hazard

The exposure assessment concluded that, for the notified and other potential activities, exposure of CGT2016 to the environment is expected to be low. Potential environmental hazards were assessed for eight points (toxicity, horizontal gene transfer, gene transfer through hybridization, interactions with other organisms, vectors of disease agents, biogeochemical cycling, habitat, biodiversity). The potential hazards of the CGT2016 to the assessment endpoints were reported to range from negligible to low.

An overall degree of uncertainty was not assigned for the final conclusion of the Science Advisory Report, because any assigned overall level of uncertainty would not reflect all outcomes/endpoints considered. The nuances of uncertainty ratings are clearly laid out in the two Risk Assessment documents.

Discussion

There was discussion on whether the eight hazard points should be combined on the Risk Assessment matrix graph (Figure 1) to establish risk. It was agreed to use eight simple hazard points on the Risk Assessment matrix graph.

It was agreed to remove the risk summary table as the final hazard assessment no longer conclude a single hazard assessment, but rather 8 individual assessments.

The discussion turned toward the colour levels indicated on the risk (hazard x exposure) matrix used in the draft environmental risk assessment (see Figure 1). A new version of the matrix (not shown) had been put forward, but because changing the matrix was beyond the scope of the discussion at this juncture in the CSAS process, the committee decided to use the original matrix that was used in the AquAdvantage risk assessment (as per Figure 1).

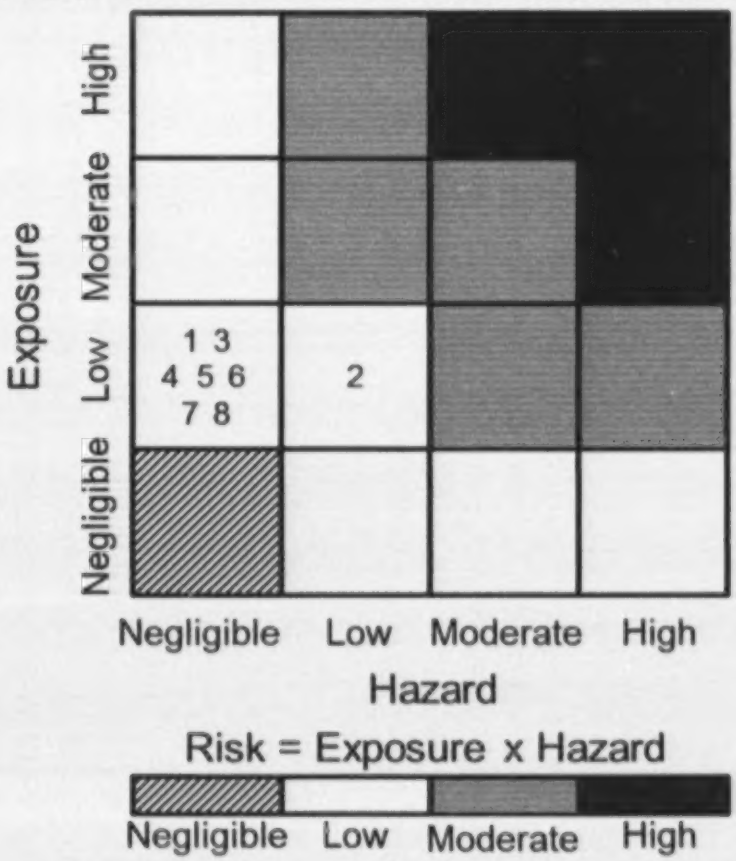

Figure 1. Risk matrix and colour scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment. Risk assessments associated with assessed hazard components at the assessed exposure are identified by number: 1) through environmental toxicity; 2) through horizontal gene transfer; 3) through interactions with other organisms; 4) through hybridization; 5) as a vector of disease; 6) to biogeochemical cycling; 7) to habitat; 8) to biodiversity.

Consensus

After the summary discussion, participants reached consensus on the following conclusions:

- The risk matrix used during the AquAdvantage risk assessment process would be adopted for this current notification (see Figure 1).
- Instead of attempting to combine hazard rankings to obtain a single overall ranking, each endpoint should be treated independently, to transparently reflect the variation in hazard and uncertainty ratings.
- Based on the risk assessment and previous discussion, the overall environmental risk associated with the import, introduction, notified use, as well as potential unintended uses of CGT2016, was concluded to be low (see Figure 1). The overall uncertainty level and any caveats to the final conclusions are to be addressed in the Science Advisory Report and revised risk assessments.

FINAL CONCLUSIONS ON RISK ASSESSMENT

Reviewers reached consensus on the final conclusions of low risk with varied levels of uncertainty ranging from negligible to moderate due to lack of consistent results in studies, lack of data from the notified or surrogate organism, and/or reliance on expert opinion and anecdotal information for both the Environmental Risk Assessment and the Indirect Human Health Risk Assessments. Overall, conclusions were that CGT2016 would not qualify as "CEPA toxic" to the environment or indirect human health.

SCIENCE ADVISORY REPORT DEVELOPMENT

The Science Advisory Report is a summary of science advice for management and for ECCC and HC. This particular Science Advisory Report is a combination of two working documents that comprise the environmental risk assessment and the indirect human health risk assessment for the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra for use in the ornamental aquarium trade in Canada. A draft of the Science Advisory Report was reviewed by all participants for the purpose of commentary and real-time edited as a group for consensus. The main task during this portion of the meeting was to write and complete the Summary section of the Science Advisory Report. Bullets were developed to explain the purpose and procedure of this risk assessment process, and a summary of the key conclusions of both risk assessments, as well as the overall conclusion on risk.

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APPENDIX 1: TERMS OF REFERENCE

ENVIRONMENTAL AND INDIRECT HUMAN HEALTH RISK ASSESSMENTS OF THE GLOFISH® ELECTRIC GREEN® TETRA AND GLOFISH® LONG-FIN ELECTRIC GREEN® TETRA

National Peer Review – National Capital Region

September 12-14, 2017

Ottawa, Ontario

Chair: Gilles Olivier

CONTEXT

The *Canadian Environmental Protection Act*, 1999 (CEPA 1999), administered by Environment and Climate Change Canada (ECCC) and Health Canada (HC), is the key authority for the Government of Canada to ensure that all new substances, including living organisms, are assessed for their potential harm to the environment and human health. The New Substances Notification Regulations (Organisms) [NSNR (Organisms)] under CEPA 1999 prescribe the information that must be provided to ECCC prior to the import to or manufacture in Canada of new living organisms that are animate products of biotechnology, including fish products of biotechnology.

ECCC and HC are responsible for conducting the **CEPA risk assessment** to evaluate whether the notified fish product of biotechnology is "CEPA toxic" in accordance with Section 64 of CEPA 1999: where a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
- constitute or may constitute a danger to the environment on which life depends; or
- constitute or may constitute a danger in Canada to human life or health.

Fisheries and Oceans Canada (DFO), ECCC and HC signed a Memorandum of Understanding respecting the implementation of the NSNR (Organisms) for new living fish products of biotechnology. DFO assists in implementing the NSNR (Organisms) by providing science advice (a Science Advisory Report) based on an environmental risk assessment for fish products of biotechnology, and, with the support of HC, on the indirect human health risk assessment for fish products of biotechnology. In addition, DFO will recommend any necessary measures to manage risks, if required.

Based on the environmental and indirect human health risk assessments (working papers), DFO provides science advice to ECCC and HC in support of their CEPA risk assessment and decision making process for products of biotechnology that have been notified under the NSNR (Organisms).

OBJECTIVES

The objective of this Science National Peer Review Process is to peer review the draft Environmental and Indirect Human Health Risk Assessments of the GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra and any recommended measures to manage risks, if required, and provide relevant science advice (i.e., the Science Advisory Report) on the assessments and recommendations.

Working papers to be reviewed will include:

- Environmental Risk Assessment of the GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra; and
- Indirect Human Health Risk Assessment of the GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra

The DFO environmental risk assessment will include consideration of potential risks to fish, fish habitat and the environment in general. The HC indirect human health risk assessment will not consider potential risks related to human consumption, but will consider potential risks from environmental exposures to the living organism such as risks from exposure to toxins and allergens and the transmission of zoonotic diseases.

The CSAS Process will evaluate the conclusions, rankings and recommendations of the draft risk assessments and any recommended measures to manage risks, including the weight of scientific evidence, quality of data, identified gaps and associated uncertainties of the:

- Characterization of GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra;
- Environmental and indirect human health exposure: characterization and assessment;
- Environmental hazard: characterization and assessment;
- Indirect human health hazard: characterization and assessment;
- Environmental risk assessment; and
- Indirect human health risk assessment.

EXPECTED PUBLICATIONS

- Science Advisory Report
- Research Document(s)
- Proceedings

The publications will be subject to third party confidential business information claims by the regulatory proponent and nondisclosure requirements in accordance with the *Access to Information Act* and the *Canadian Environmental Protection Act*, 1999.

PARTICIPATION

- Fisheries and Oceans Canada (Ecosystems and Oceans Science Sector; Pacific Region; Central and Arctic Region, Gulf Region)
- Environment and Climate Change Canada and Health Canada
- Academia
- Other invited experts

APPENDIX 2: AGENDA

Agenda of the CSAS Science National Peer-Review Process
Environmental and Indirect Human Health Risk Assessments of the GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra
September12-14, 2017
Innovative Professional Offices
440 Laurier Ave. W, 2nd Floor
Ottawa, ON K1R 7X6

DAY 1 – TUESDAY, SEPTEMBER 12

8:30 - 8:45	Welcome and introductions *(Gilles Olivier)*
8:45 – 9:00	Introduction to CSAS Science National Peer-Review Process *(Gilles Olivier)*
9:00 – 9:30	Context: Regulatory, risk assessment, proposed use *(Colin McGowan)*
9:30 – 10:30	Characterization of GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra *(Rosalind Leggatt)*
10:30 - 10:45	Break
10:45 – 11:30	Indirect human health exposure assessment *(Kassim Ali)*
11:30 – 12:00	Consensus: Indirect human health exposure assessment *(All)*
12:00 – 1:00	Lunch
1:00 – 2:00	Indirect human health hazard assessment *(Stephen Dugan)*
2:00 – 2:30	Consensus: Indirect human health hazard assessment *(All)*
2:30 – 2:45	Break
2:45 – 3:45	Indirect human health risk assessment *(Kassim Ali)*
3:45 - 4:15	Consensus: Indirect human health risk assessment *(All)*
4:15 – 4:30	Summary of Day 1 and adjournment *(Gilles Olivier)*

DAY 2 – WEDNESDAY, SEPTEMBER 13

8:30 – 9:00	Review and summary of conclusions so far *(Gilles Olivier)*
9:00 – 10:00	Characterization of the receiving environment *(Colin McGowan)*
10:00 – 10:30	Break
10:30 – 11:30	Environmental exposure assessment *(Colin McGowan)*
11:30 – 12:00	Consensus: Environmental Exposure Assessment *(All)*
12:00 - 1:00	Lunch
1:00 – 2:00	Environmental hazards assessment *(Rosalind Leggatt)*
2:00 – 2:30	Consensus: Environmental hazard assessment *(All)*
2:30 – 2:45	Break
2:45 – 3:45	Environmental risk assessment *(Colin McGowan, Rosalind Leggatt)*
3:45 – 4:15	Consensus: Environmental risk assessment *(All)*
4:15 – 4:30	Summary of Day 2 and adjournment *(Gilles Olivier)*

DAY 3 – THURSDAY, SEPTEMBER 14

8:30 – 8:45	Review and summary of conclusions so far *(Gilles Olivier)*
8:45 – 9:00	Proposed risk management measures, if needed *(Colin McGowan)*
9:00 – 10:30	Science Advisory Report development *(All)*
10:30 – 10:45	Break
10:45 – 12:00	Science Advisory Report development *continued (All)*
12:00 – 12:15	Final Consensus *(All)*
12:15 – 12:30	Conclusions and adjournment *(Gilles Olivier)*
12:30	End of meeting

APPENDIX 3: MEETING PARTICIPANTS

Table 1. Participants of the CSAS Science National Peer-Review Process Environmental and Indirect Human Health Risk Assessments of the GloFish® Electric Green® Tetra and GloFish® Long-Fin Electric Green® Tetra

Name	Affiliation
Gilles Olivier	Chair; Fisheries and Oceans Canada
Jay Parsons	Fisheries and Oceans Canada
Colin McGowan (Co-author)	Fisheries and Oceans Canada
Rosalind Leggatt (Co-author)	Fisheries and Oceans Canada
Neville Johnson (Co-author)	Fisheries and Oceans Canada
Stephen Dugan (Co-author)	Health Canada
Kassim Ali (Co-author)	Health Canada
George Arvanitakis	Health Canada
Arash Shahsavarani	Environment and Climate Change Canada
Jim Louter	Environment and Climate Change Canada
Marten Koops	Fisheries and Oceans Canada
Sherry Walker	Fisheries and Oceans Canada
Ingrid Burgetz	Fisheries and Oceans Canada
Bob Devlin	Fisheries and Oceans Canada
Shauna Baillie	Fisheries and Oceans Canada
Tricia Gheorghe	Fisheries and Oceans Canada
Sophie Foster	Fisheries and Oceans Canada
Anne-Margaret MacKinnon	Fisheries and Oceans Canada
Sandra Noble	University of Ottawa
Tillman Benfey	University of New Brunswick
Richard Renlund	St Michael's Hospital